AD-A177 915 MOLECULAR AND BIOLOGICAL PROPERTIES OF AN IMMUNOPOTENTIATING COMPLEX POLY..(U) CALIFORNIA UNIV BERKELEY DEPT OF MICROBIOLOGY AND IMMUNOLOGY.. 1/1

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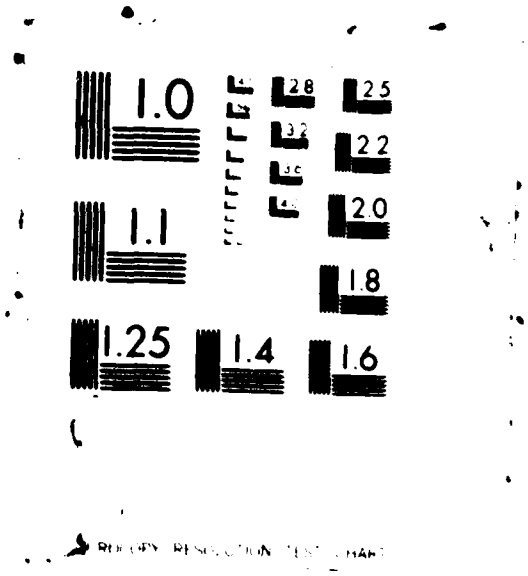
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A species of the gliding bacteria Cytophaga produces an exopolymer, designated GBA, that potentiates immune reactions. We showed previously that GPA augments primary immune responses in vitro, causes P lymphocytes to proliferate and secrete immunoglobulin, and induces macrophages to secrete Interleukin 1. GPA is prepared from culture supernatants of Cytophaga. Chemical analysis of these molecules indicates that they are large particulate polymers of amino sugars.

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In the period covered by this report we examined GPA for additional immunomodulatory activities. We found that GPA stimulates cultured human peripheral blood cells to secrete tumor necrotizing factor alpha and that it blocks the suppressive effects of anti-IgM on the pre-P cell line WEHI 231. We also found that GPA augments endogenous colony forming units in radiation-treated mice and that it protects mice from otherwise lethal infectious doses of selected strains of F. coli. Other experiments showed that GBA is an immunological

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Block 19, continued:

adjuvant *in vivo*. Primary humoral responses of mice immunized with protein antigens in combination with GBA were significantly higher than those of mice immunized with antigen alone. Moreover preliminary experiments showed that GBA augments antigen-specific immunological memory.

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Molecular and Biological Properties of an Immunopotentiating Complex Polysaccharide Adjuvant Produced by a Gliding Bacterium

INTRODUCTION AND BACKGROUND

In our previous report we described the purification and initial chemical characterization of a high molecular weight, complex polysaccharide synthesized by a newly described species of Cytophaga. The polymer enhances immune responses of cultured mammalian cells and for this reason we have designated it gliding bacterium adjuvant (GBA). GBA is secreted or shed into the growth medium. The physical and chemical properties of GBA were summarized (1). Based on these results, we hypothesized that GBA is composed of amino sugar polymers, containing neither primary amines nor viscinyl hydroxyl groups. Consistent with this interpretation, GBA reacts like other amino sugars in the phenol-sulfuric hexose assay. We reported previously that purified GBA functions not only as an in vitro adjuvant but also stimulates B cells to proliferate and secrete immunoglobulin polyclonally. In addition, GBA causes macrophages to release Interleukin 1 (IL-1), a hormone of central importance in immune and inflammatory reactions.

GBA as an Adjuvant, In Vivo

The ability of GBA to induce IL-1 and augment primary immune responses in vitro suggested that it might function as an adjuvant in vivo. We examined this possibility by injecting sets of BALB/c mice with antigen, with or without GBA, and measuring antigen-specific humoral responses 9-11 days later by solid-phase ELISA. The results of one such experiment using cytochrome C as the test antigen showed that GBA augmented primary responses by 5-75 fold compared to those of controls that received antigen alone. Similar results have been obtained with lysozyme and ovalbumin.

GBA Boosts Amnestic Responses to Lysozyme

Although the above studies were promising, they were not designed to address an important aspect of adjuvanticity, namely the ability to augment memory responses. The following experiment was performed to determine whether GBA affects secondary responses to lysozyme.

Two sets of 12 mice were injected with lysozyme or lysozyme + GBA. Mice were bled 12 days later and then rested for 6 weeks. All mice were then re-bled and re-injected with antigen only. Seven days later mice were bled and their sera were tested for antigen-specific responses to ELISA.

None of the 12 mice that received only antigen during the initial phase of the experiment mounted a secondary response: the titers before boosting remained unchanged after boosting. In contrast, 7 of 12 mice given GBA during their priming showed a 5-25 fold increase in titer following the second injection (with antigen only). This preliminary experiment indicates that GBA increases immune memory as well as increasing primary responses. If confirmed by further study, this finding supports our contention that GBA has potential application for use in human and veterinary vaccines.

GBA BOOSTS NON-SPECIFIC RESISTANCE TO INFECTION AND IRRADIATION

Infectious Challenge Studies

In collaboration with Dr. K. T. Chong of CETUS Corporation we examined whether GBA could affect the survival of mice given a lethal dose of E. coli derived from human clinical isolates. A series of experiments were performed to examine the effects of dose, kinetics and route of administration of GBA. In the first set of experiments, outbred CD_1 mice (6 mice/group) were injected i.p. with various doses of GBA two days prior to an i.p. injection of bacteria. Survival was scored 7 days following injection. The results show extensive protection occurred with doses as low as 70 nanograms.

Table 1

Protective Effects of GBA on Subsequent Inoculations of E. coli in CD_1 Mice

Micrograms GBA/Mouse	% Survival Expt. #1	% Survival Expt. #2
Saline control	16.7	16.7
17.5	100	83.3
7.0	66.7	83.3
0.7	100	83.3
0.07	66.7	66.7

In the second experiment, GBA was administered at various times relative to that at which mice were inoculated with E. coli; -4 hours, -1 hour and +1 hour were examined. As shown in Table 2, protection occurred only with mice that received GBA 4 hours before inoculation with E. coli.

Table 2

Effects of the Time of GBA Administration in Relation to E. coli Inoculation on the Capacity of GBA to Protect CD_1 Mice

Time* (hours)	% Survival at 7 Days
Saline control	20
GBA -4	60
-1	10
+1	20

* Time of administration time of GBA (7.0 ug/mouse, i.p.) relative to time of inoculation with E. coli (10 mice/group).

In the third set of experiments, mice were treated with GBA 18 and 4 hours before they were inoculated with E. coli. The GBA was administered intraperitoneally to one set of mice and subcutaneously to another. Results are shown in Table 3. GBA was effective at both times when administered intraperitoneally but had no effect when given subcutaneously.

Table 3

Effects of Timing and Route of Administration of GBA on Survival of CD_1 Mice Inoculated with E. coli

Time	Route	% Survival
-18 hr	i.p	100
-4 hr	i.p	80
-18 hr	s.c.	20
-4 hr	s.c	20

Ten mice were used for each group; 7.0 ug of GBA was administered to each mouse; 20% of controls that received only saline survived the infection.

These studies indicate that small doses of GBA can protect mice against lethal bacterial challenges when administered i.p or s.c. 4-48 hours prior to infection. They indicate that GBA is an effective nonspecific adjuvant and therefore may be useful for prophylaxis. Thus far the data indicate that GBA is ineffective when given after inoculation. We plan to expand this study by (1) investigating broader dose ranges of GBA and E. coli and (2) examining resistance to viral infections and infections with Gram-positive bacteria.

Radioprotection Studies

In collaboration with Dr. Myra Patchen of the Armed Forces Radiobiology Research Institute, we are examining the potential of GBA to stimulate hematopoiesis following radiation. In these studies 50 ug of GBA was injected either intravenously or intraperitoneally into mice approximately 20 hours prior to irradiation. The radiation dose (650 rads) partially destroys the pluripotent hematopoietic stem cell population. The surviving stem cells proliferate; if they are numerically sufficient and adequately stimulated, they can reconstitute the host. The proliferation of these stem cells can be detected as gross nodules on the spleen surface (12 days post-irradiation). The nodules are referred to as endogenous colony-formin units (F-CFU) indicating that they arose from surviving endogenous pluripotent stem cells. As shown in Table 4, GBA enhances hematopoietic recovery of radiation-injured mice.

Table 4

Effects of GBA on Hematopoietic Injury of Sublethally Irradiated Mice

Injection	Endogenous-CFU
Control	6
i.v. - 50 ug GBA	15
i.p. - 50 ug GBA	25

These results indicate that GBA may either have induced stem cell proliferation in the period prior to irradiation allowing more stem cells to survive or that GBA enhanced the post irradiation repopulation by providing a significant proliferative stimulus to the surviving stem cells. GBA may have acted directly or indirectly by stimulating the production of endogenous hormones such as IL-1 or colony stimulating factors which in turn may have acted on stem cells.

GENERATION OF TUMOR NECROSIS FACTOR (TNF)

We are examining whether GBA, by itself, or in combination with other factors, can induce different cell types to secrete TNF-alpha. In collaboration with Dr. Michael Palladino of Genentech, Inc., supernatants generated under a variety of conditions were assayed directly for TNF by an ELISA, using a TNF-alpha-specific monoclonal antibody and recombinantly-derived TNF-alpha as a standard. Freshly prepared human peripheral blood mononuclear cells or the human promyelocytic cell line HL-60 were treated with GBA or phorbol ester (10 ng/ml) as shown.

Table 5

Synergistic Effect of GBA on the Induction of TNF

by HL-60 Cells Treated with PMA

Target Cell	Inducers		TNF-alpha (pg/ml)
	PMA	GPA (ug/ml)	
HL-60	0	0	114
	0	1	118
	0	10	127
	0	100	93
	+	0	1544
	+	1	1773
	+	10	2296
	+	100	3080

Table 6

Induction of TNF by Peripheral Blood Leukocytes Treated with GBA

Human PBL	GBA (ug/ml)	TNF-alpha (pg/ml)
Donor #1	0	70
	2	165
	50	217
Donor #2	0	<44
	2	73
	50	131

These results indicate that GPA weakly induces the release of TNF by cultured normal human leukocytes and weakly synergizes with phorbol to stimulate release of TNF by HL-60 cells. We are repeating these preliminary experiments and are attempting to define optimal conditions.

GPA BLOCKS THE IMMUNOSUPPRESSIVE EFFECTS OF ANTI-IgM ON THE PRE-B CELL LINE WEHI 231

In collaboration with the laboratory of Dr. A. DeFranco, we have examined GBA and other products of bacterial origin (lipopolysaccharide and peptidoglycan) for their effects on the pre-B cell line WEHI-231 (2). Each of the bacterial products temporarily blocks the anti-proliferative effects of anti-IgM. Anti-IgM is thought to mimic antigen triggering of normal immature B cells.

Studies of the underlying pathways of signal transduction indicate that GBA and the other bacterial substances probably share a common pathway in these cells. These studies have not identified the mechanisms by which the bacterial products initiate signalling. We are pursuing this question by determining if GBA binds to the WEHI 231 cells by virtue of specific surface receptors. Occupation of such receptors may in turn initiate signalling.

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